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Antimicrobial property and phytochemical study of ginger found in local area of Punjab, Pakistan

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ABSTRACT

The aim of study is to identify the antimicrobial property of ginger. Phytochemical screening of chloroform plant extract showed presence of different chemicals. In this study we used Cultures of *E. Coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus faecalis* to identify the antimicrobial strength. Effectiveness of ginger against different conditions attributed to its different constituents (volatile oils, shogaols, Gingerols and diarylheptanoids) that show their therapeutic efficacy by modulating the genetic or metabolic activities of our body. In this study, we performed phytochemical evaluation and antimicrobial assay of ginger root extract which were available in our local farms of Lahore. Ginger possesses a noticeable antimicrobial activity which was confirmed by checking the susceptibility of different strains of bacteria and fungus by measuring the zone of inhibition. In the light of several socioeconomic factors of Pakistan mainly poverty and poor hygienic condition, present study encourages the use of spices as alternative or supplementary medicine to reduce the burden of high cost, side effects and progressively increasing drug resistance of pathogens.

Key Words: genus Zingiber, antimicrobial, phytochemical, rhizome.

INTRODUCTION

Rhizome or root part of ginger (genus Zingiber) (figure 1) is extensively employed in medicine for the management of different diseased conditions like nausea, vomiting, motion sickness, gastrointestinal ulcers, diabetes, fever, arterial tension, rheumatoid arthritis, dry mouth/xerostomia, cancer, migraine headache, sore throat, minor respiratory ailments. *Zingiber officinale* Roscoe, commonly known as Ginger, belongs to Zingiberaceae family (Kadnur and Goyal, 2005). Gengibre, Ancoas are the most frequently used Spanish names of Ginger (Ravindran and Babu, 2004; Maldonado, 2014) but in Pakistan, it is known as "Adrac" (Boyd, 1999).

Microbial pathogenicity and other infectious diseases have been controlled by use of commercially available antimicrobial drugs since last many years. Tremendous use of antibiotics has developed multiple drug resistance (MDR) in many bacterial pathogens. In the present study, *in vitro* antimicrobial activity of some local spices of Pakistan, that are routinely used in food, has been investigated against clinically important bacterial pathogens. South East Asia is considered as home grown land for Ginger production (Ravindran and Babu, 2004). By tradition, ginger farming is common in number of countries like Japan (Ravindran and Babu, 2004), China (Ravindran and Babu, 2004), Indonesia (Ravindran and Babu, 2004), Nigeria (Powell, 1986), India (Rahman *et al.*, 2009), Brazil (Govindarajan and Connell, 1983), Sri Lanka (MacLeod and Pieris, 1984), the Philippines (Cramb *et al.*, 2000) and Jamaica Islands (Ravindran and Babu, 2004). In Pakistan, commercial production of Ginger is not

sufficient to converge its indigenous provisions (Iqbal *et al.*, 2006) because its marketable cultivation is restricted to merely ten districts of Sindh (Panahwar, 2005). The best climate requirements for its cultivation are sandy clay (Bhosale and Shinde, 2011), semi-tropical (Chang *et al.*, 2011) and temperate zone (Newman *et al.*, 1997) with sufficient organic matter and pH of about 5.5-6.6 (Williams *et al.*, 2003).

The horizontally solid underground stem / rhizome (with elegantly covered skin) of this plant has proved to be one of the most extensively used culinary agent and spice in daily home cooking practice (Naveena *et al.*, 2004; Lantz *et al.*, 2007). Despite of its use as flavoring agent, ginger is also appreciated in ayurvedic, tibbe-e-unani (Srivastava and Mustafa, 1989), allopathic (Fessenden *et al.*, 2001), aromapathy (Shelly *et al.*, 2004) and household medicines (Sloand and Vessey, 2001). Ginger rhizome can be employed in the form of fresh paste, ginger tea (flavoring), dried powder and preserved slices (El-Ghorab *et al.*, 2010).

Ginger can be available in different commercial products like cookies, candy, teas, tinctures, sodas, jam, beer, capsule and syrup (Maxwell, 2008). The chief active constituents of ginger are Volatile oil (zingiberene, zingiberol, D-camphor), Shogaols, Diarylheptanoids, Gingerols, Paradol, Zerumbone, 1-Dehydro-(10) gingerdione, Terpenoids and Ginger flavonoids (Baliga *et al.*, 2012). Shogaols and Gingerols are responsible for ginger's pungency (Suekawa *et al.*, 1984). Ginger has wide range of biological activities that are attributed to its active constituents (Shukla and Singh, 2007); some of these are listed in table 1.

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Figure 1: Ginger.

MATERIALS AND METHODS

Plant material

Ginger rhizomes were collected from a farm near Lahore, Pakistan in March 2014. Collection was performed by pulling plants out of the soil and transferring them into sealable plastic bags.

Sample preparation

The rhizomes were washed to remove soil, peeled and washed again in clean water. After washing, the rhizomes were dried, powdered and submitted to successive extraction by chloroform in separating funnel at room temperature. Collect the lower layer of chloroform in conical flask and heat the solution for a while. Chloroform will evaporate from flask and add water in this and mix. The extracts further sterilized by filtration (0.22 μ m).

Phytochemical screening of extract

In order to perform the phytochemical evaluation of ginger extract, methods already described in literature

Table 1: Biological activities of ginger reported in literatures.

Active principles	Biological action	MOA	References
Gingerols, shogaols, Sesquiterpenes and monoterpenes)	For the treatment of nausea and vomiting	By anticholinergic and antiserotonin action	(Bryer, 2005)
Ethanollic extract of ginger	Hypolipidimic agent	By reducing triglycerides and LDL cholesterol and to increase HDL	(Bolanle, 2011)
[6]-gingerol	Anti-tumor property	inhibition of NF- κ B, stimulation of apoptosis and inactivation of VEGF pathways lead to antineoplastic effects	(Surh <i>et al.</i> , 1998)
6-Shogaol	Anti-inflammatory effect	Inhibition of pro-inflammatory cytokines (IL-12, TNF- α , IL-1 β , IL-8) and chemokines (RANTES, MCP-1).	(Penna <i>et al.</i> , 2003)
	Anticancer activities (e.g. breast cancer)	inhibition of cell invasion reduction of matrix metalloproteinase-9 expression	(Ling <i>et al.</i> , 2010)
Zingiberone and ethanollic extract of ginger	Anti-hyperglycemic effect	Lowering of blood glucose level by inhibition of oxidative stress and anti-inflammatory process, increase insulin sensitivity.	(Vats <i>et al.</i> , 2002; Shanmugam <i>et al.</i> , 2011)
6-shogaol, phenolic and favonoids compounds	Neuroprotector effect	by accelerating brain anti-oxidant defense mechanisms	(Shanmugam <i>et al.</i> , 2011)
Ginger extract (highly purified and standardized)	Treatment of osteoarthritis of the knee joints	By reduction of inflammatory mediators.	(Bliddal <i>et al.</i> , 2000; Altman and Marcussen, 2001)
[6]-gingerol and [6]-shogaol	anti-ulcerative effects	By Suppressing the gastric contraction, increasing mucin secretion	(Minaiyan <i>et al.</i> , 2006)
Sesquiterpenes (B-Sesquiphellandrene)	Anti-viral effect		(San Chang <i>et al.</i> , 2013)
Gingerol and shogaol	Antiplatelet activity	lower platelet thromboxane X2 and prostaglandin E2 production,	(Nurtjahja-Tjendraputra <i>et al.</i> , 2003)
Gingerol and shogaol	Hypotensive effects	lowering blood pressure by inhibition of voltage-dependent calcium channels as well as by stimulating muscarinic receptors	(Chayur <i>et al.</i> , 2005; Nicoll and Henein, 2009)
Aqueous extract of ginger	Hepato-protective effect (against the CCl ₄ , acetaminophen and lead)	By decreasing ALT, AST and ALP levels and enhancing the activities of SOD, GST, CAT and GSH levels in the liver	(Bhandari <i>et al.</i> , 2003; Bai <i>et al.</i> , 2011; Sabina <i>et al.</i> , 2011; Pratap and Indira, 2014)

Table 2: Phytochemical evaluation methods.

Tests	Description
Alkaloids test	5ml of the ginger extracts were accurately measured and transferred into a flask and stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath. Then 1ml of that filtrate was treated with few drops of Dragendorff's reagent. A color change to blue black was the evidence of presence of alkaloids.
Saponins test	5ml of the ginger extracts and 5ml distilled water in a test tube. Appearance of Frothing on shaken with water showed the presence of saponins.
Tannins test	5ml of the ginger extracts along with 100ml distilled water and filtered, then ferric chloride reagents was added, blue-black or blue green precipitate appeared which showed the presence of Tannins.
Phlobotannins test	When an aqueous extract of the test sample (ginger) was boiled with 1% hydrochloric acid, disposition of red precipitate had confirmed the presence of phlobotannins.
Flavonoids test	When 5ml of diluted ammonia solution was added to aqueous filtrate of the test sample (ginger extract) followed by the addition of concentrated H ₂ SO ₄ , a yellow coloration was observed which determined the presence of flavonoids.
Cardiac glycosides (keller-killiani test)	When 5ml of the ginger extracts dissolved in 2ml glacial acetic acid containing a drop of ferric chloride solution was underplayed with 1ml concentrated H ₂ SO ₄ . A brown ring appeared at interface indicated adeoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a green ring may form just gradually spread throughout this layer.
Steroids test	When 2 ml of acetic anhydride was added to 0.5 g of ginger extract and 2 ml of sulphuric acid was added by the sides of the test tube a color change was observed to violet or blue-green which showed the presence of steroids
Terpenoids test	When 2 ml of chloroform was added to 1ml of the extract, and Conc. H ₂ SO ₄ (3 ml) was added to form a layer, a reddish brown coloration at the interface indicated the presence of terpenoid.

with slight modification, were used for the screening of alkaloids, steroids, phlobotannins, flavonoids, glycosides, saponins, tannin and terpenoids (table 2).

Antimicrobial assay

The antimicrobial activity of ginger extract against various human pathogens was determined by agar diffusion method.

Four bacterial cultures were used, named *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Streptococcus faecalis*. Soybean-Casein Digest Agar (SCDA) was used as the culture media. Sterile saline tubes (5ml), 70% IPA, sterile test tubes, sterile petri-plates, 250ml conical flasks, Borer, Micropipette, sterile tips, sterile inoculating loop, autoclave, shaker, oven (32-35°C), laminar air flow hood, incubator, weighing balance and Vernier caliper were used for the assay.

From cultures of *E. Coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus faecalis*, slants were made and incubated at 32°C for 24-48 hrs. Loops from each slant culture were transferred separately in 5 ml sterile saline solution tube to prepare suspension of each culture. These suspensions were transferred separately to 150 ml sterile SCDA when cooled to a temperature of 40-45°C. Each conical flask having SCDA and culture suspension was shaken to allow uniform distribution of microbial cells in medium. After shaking each SCDA medium with culture suspension was poured into four plates, labeled and allowed to solidify. After solidification of the medium in plates, the wells were cut in each plate using sterile borer. 0.1 ml of 10% (w/v) ginger extract to be tested was poured into different wells and the plates were incubated at 32°C for 24 hrs. After incubation the plates were observed for the presence of zone of inhibition. If present, the diameter of zone of inhibition was measured with the help of vernier caliper.

Two fungi cultures were also used, namely *Candida albicans* and *Aspergillus niger*. Sabouraud dextrose agar

(SDA) was used as culture media. Sterile saline tubes (5ml), 70% IPA, sterile test tubes, sterile Petri plates, 250ml conical flasks, Borer, Micropipette, sterile tips, sterile inoculating loop, autoclave, shaker, oven (32-35°C), laminar air flow hood, incubator, weighing balance and Vernier caliper were used for the assay.

From cultures of *Candida albicans* and *Aspergillus Niger*, slants were made and incubated at 22°C for 24-48 hrs. A loop from each slant culture was transferred separately in 5 ml sterile saline solution tube to prepare suspension of each culture. This 5 ml suspension of each culture were transferred separately to 150 ml sterile SDA when cooled to a temperature of 40-45°C. Each conical flask having SDA and culture suspension was shaken to allow uniform distribution of microbial cells in medium. After shaking each SDA medium with culture suspension was poured in three plates, labeled and allowed to solidify. After solidification of the medium in plates, the wells were cut in each plate using sterile borer. 0.1 ml of 10% (w/v) extract of ginger plant to be tested was poured into different wells. The plates were incubated at 22°C for 24 hrs. After incubation the plates were observed for the presence of zone of inhibition. If present, the diameter of zone of inhibition was measured with the help of vernier caliper.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening of chloroform plant extract showed presence of alkaloid, phlobotannins, flavanoids, glycosides, saponins, tannin and terpenoids and absence of steroids (table 3).

Antimicrobial assays

The findings of the present study revealed that *Zingiber officinale* contain potent antimicrobial property against tested microbes. The antimicrobial activity of the ginger extracts (chloroform extract) was initially evaluated by

Table 3: Qualitative phytochemical analysis of crude extract of *Zingiber officinale* (Ginger roots).

Bioactive principle	Chloroform extract of ginger	Methanol extract of ginger
Alkaloids	+++	+++
Tannins	++	++
Glycosides	++	++
Saponins	+++	+++
Steroids	-	-
Flavonoids	++	++
Terpenoids	+	+
Phlobotannins	+	+

Key = +++ abundantly present, + fairly present, ++ moderately present, - absent

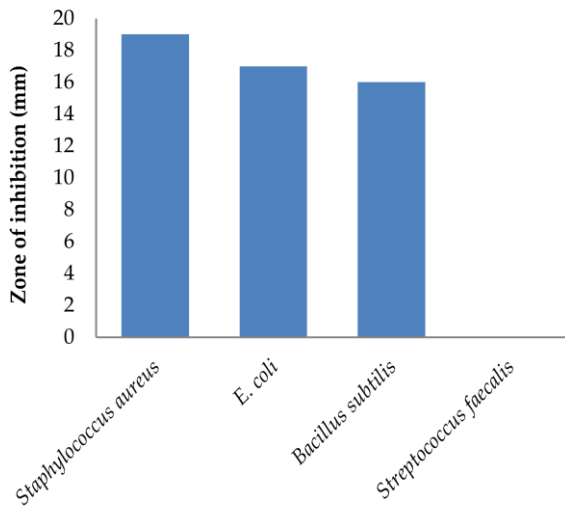


Figure 2: Zone of inhibition of bacteria - ginger extract has shown maximum antimicrobial activity towards *S. aureus*.

Table 4: Degree of sensitivity of bacteria plus fungi against ginger extract by measuring zone of inhibition.

Microorganisms	NCIM NO	Zone of inhibition (mm)
Bacteria <i>Staphylococcus aureus</i>	2079	19
<i>E. coli</i>	2065	17
<i>Bacillus subtilis</i>	2063	16
Fungi <i>Streptococcus faecalis</i>	5024	-
<i>Candida albicans</i>	3471	-
<i>Aspergillus Niger</i>	1196	20

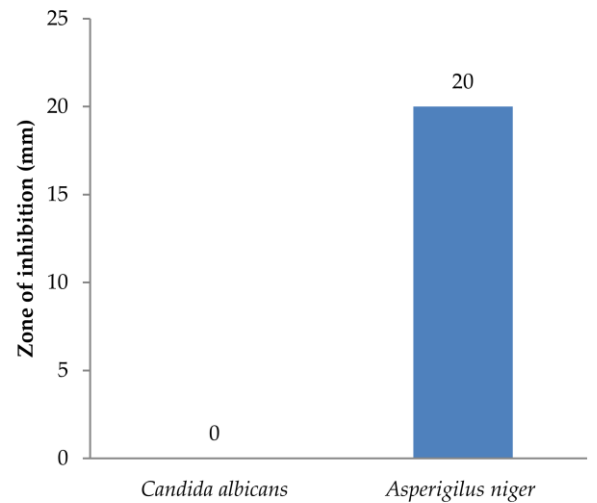


Figure 3: Zone of inhibition of fungi - ginger extract has shown maximum antifungal activity towards *Aspergillus niger*.

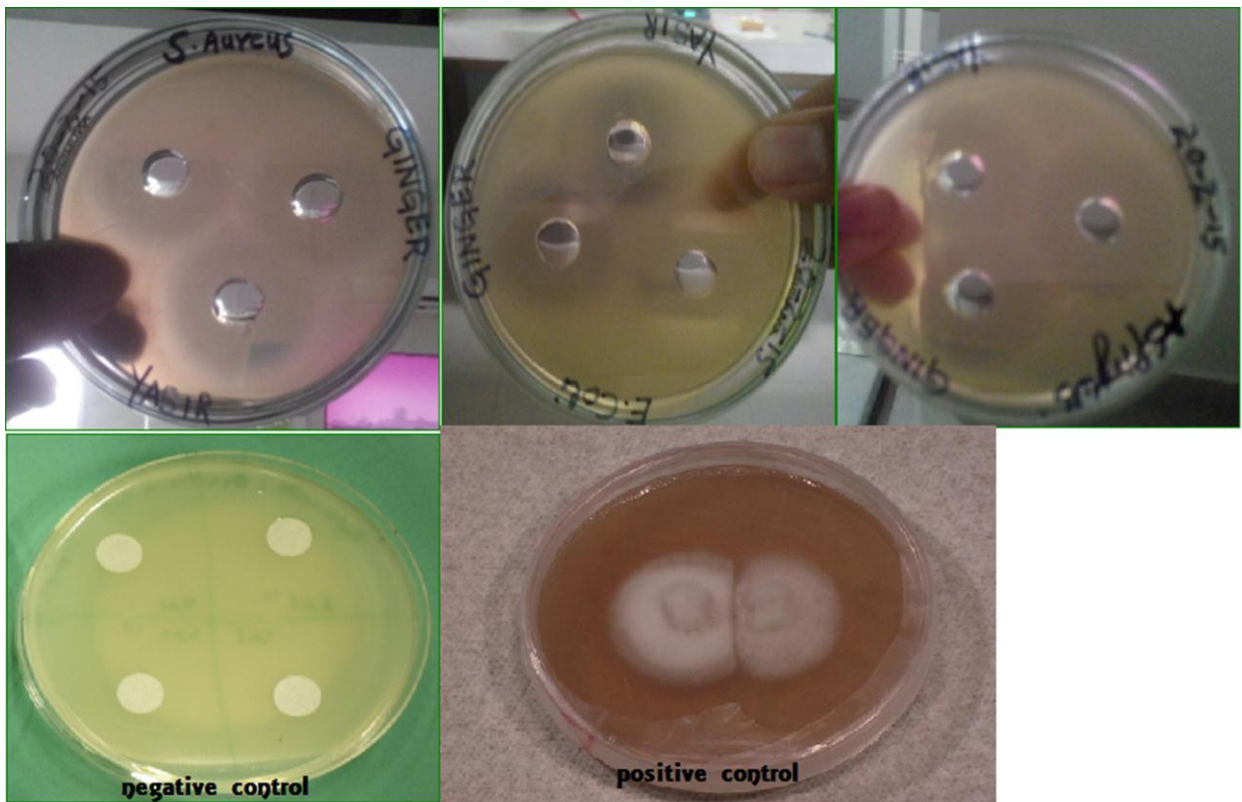


Figure 4: Zone of inhibition on Nutrient agar and Sabouraud Dextrose Agar.

agar diffusion method using four strains of pathogenic bacteria *Escherichia Coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis* and two stains of fungi *Candida albicans* and *Aspergillus Niger*. These extracts exhibited antimicrobial activity (table 4, figure 2-4).

CONCLUSION

The results of our experiments showed that different bacterial species exhibited different sensitivities towards the extract of ginger. Today, most pathogenic organisms are becoming resistant to antibiotics. To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Most of the spices extracted either in water or in organic solvents have biologically active compounds, which can be used in the synthesis of potent drugs. Thus spices, which are normal ingredients of our routine food preparations, can provide protection to a certain extent against our natural enemies like bacterial pathogens

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